The opinion in support of the decision being entered today is not binding precedent of the Board.

Paper 19

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte YASUSHI **KOHNO**

Appeal No. 2004-0035
Application No. 10/007,186[1]

MAILED

SEP 28 2004

PAT. & T.M. OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

ON BRIEF

Before: McKelvey, Senior Administrative Judge, and TORCZON and SPIEGEL, Administrative Patent Judges.

SPIEGEL, Administrative Patent Judge.

ORDER - - VACATE AND REMAND

I. Introduction

Our review of the record leads us to conclude that this case is not in condition for a decision on appeal. Accordingly, we vacate the examiner's rejections of claims 1-3, which are all of the claims pending in this application,[2] and remand the application to the examiner for further action not inconsistent with this opinion.

[1] Application for patent filed 5 November 2001. The real party-in-interest is AGRITECNO YAZAKI CO., LTD.

[2] The examiner did not enter an AMENDMENT DURING APPEAL filed 4 August 2003.

II. Findings of fact (FF)

The following findings of fact are supported by a preponderance of the evidence.

A. Abbreviated Procedural History of Application 10/007,186[3]

1. Application 10/007,186 was filed 5 November 2001 (Paper 1).

2. A first Office action on the merits was issued 22 July 2002 (Paper 4).

3. The examiner rejected original claim 1, the sole original claim, under 35 U.S.C. § 102 as anticipated by either (a) Corbineau et al. ("Corbineau") or (b) U.S. Patent 6,107,051 granted to Job et al. ("Job") 22 August 2000 (Paper 4, pp. 2-3).

4. Although the examiner cited Corbineau as "Acta Horticulturae (No. 267); p. 191-197; Effects of priming on the germination of Valerianella olitoria seeds in relation with temperature and oxygen; Corbineau, F.; Come, D.; ISSN: 0567-7572; ISBN: 90-6605-134-5; 1990," the examiner appears to have relied solely on the "attached abstract" instead of the full text Corbineau article (Paper 4, p. 2).

5. A copy of the "attached [Corbineau] abstract" is provided as Exhibit 1001.

6. The examiner also made of record, but did not rely upon, four other references considered to be "pertinent to applicant's disclosure", i.e., "U.S. Patent No. 5,910,281 [Kohno et al.], U.S. Patent No. 4,735,017 [Gago et al.], Australia Patent AU8310760 [Coles], and Romania Patent RO113935B [Badiu et al., hereinafter "Badiu"]" (Paper 4, p. 3).

7. In response, appellant amended claim 1, added new claims 2-3, and traversed the rejections based on Corbineau and Job (Paper 5, filed 17 September 2002).

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[3] This is not a complete prosecution history of the involved application.

8. As to Corbineau, appellant argued, in relevant part, that the Corbineau abstract made of record by the examiner was inconclusive because of its lack of detail, i.e., it was unclear what Corbineau actually taught (Paper 5, pp. 4-5).

9. The examiner then finally rejected the currently pending claims on 26 November 2002 under 35 U.S.C. § 103 as follows (Final Rejection, "FR," Paper 6):

(a) claim 1 "over Romanian Patent RO113935B to Badiu in view of Journal of New Seeds, *Seed Soaking Damage in Some Grain Legumes*, Mike Smith, Nov. 2000" (Paper 6, p. 2);

(b) claims 2 and 3 "over Romanian Patent RO 113935B to Badiu et al as applied to claim 1 above, and further in view of *Starting Plants from Seed*, Evans et al, NC State University, 1999, section on Light" (Paper 6, pp. 2-3);

(c) claim 1 "over U.S. Patent No. 6,107,051 to Job et al. in view of Journal of New Seeds, *Seed Soaking Damage in Some Grain Legumes*, Mike Smith, Nov. 2000" (Paper 6, p. 3); and

(d) claims 2 and 3 "over U.S. Patent No. 6,107,051 as applied to claim 1 above, and further in view of *Starting Plants from Seed*, Evans et al, NC State University, 1999, section on Light" (Paper 6, p. 4).

10. In setting forth the final rejection, the examiner referred to "Badiu et al English abstract" and "Smith abstract last sentence" (Paper 6, p. 2).

11. The Romanian patent to Badiu was already of record.

12. The record contains a four page table of "Contents" from the Journal of New Seeds, Vol. 2, No. 3 (2000), including title, author, abstract and keywords for each

article in the volume.

13. The table of "Contents" (attached as Exhibit 1002) lists the second article in the volume as "Seed Soaking Damage in Some Grain Legumes" by Demosthenis Chachalis and Mike L. Smith said to begin on page 27.

14. The article by Chachalis et al. appears to be the underlying document for the Smith abstract relied upon by the examiner.

15. The examiner made no reference to either the Corbineau article or the Corbineau abstract in the final rejection (Paper 6).

16. A notice of appeal was filed 27 January 2003 (Paper 7).

17. Appellant also filed a request to amend the title of the involved application along with remarks requesting reconsideration of the final rejection on 27 January 2003 (Paper 8).

18. The examiner requested a translation of the Badiu Romanian language patent on 11 February 2003. ("Request Form for Translation," attached as Exhibit 1003).

19. The examiner denied entry of the amendment to the title and denied making any changes in the final rejection (Paper 9, issued 13 February 2003).

20. Appellant's Brief On Appeal ("Brief," Paper 12) was filed 26 March 2003.

21. The Examiner's Answer ("Answer," Paper 13) was issued 30 May 2003.

22. The examiner withdrew the rejection of claim 3 under 35 U.S.C. § 103 over Job; all other rejections under § 103 were maintained (Paper 13, p. 3).

23. Appellant's Reply Brief ("Reply," Paper 15) was filed 4 August 2003 with an "<u>AMENDMENT DURING APPEAL</u>," (Paper 14).

24. The examiner refused entry of the amendment and notified appellant that the application was being forwarded to the Board ("Letter" dated 22 August 2003, Paper 16).

25. The application was received at the Board on 26 August 2003 with only an abstract of the Badiu Romanian patent (see "ORDER RETURNING UNDOCKETED APPEAL TO EXAMINER," issued 29 August 2003, Paper 17).

26. The application was returned for the examiner to provide "a full text translation of Romanian Patent No. 113,935, ... and for such further action as may be appropriate" (Paper 17, p. 2).

27. The application was returned to the Board on 1 September 2003 and assigned Appeal No. 2004-0035 ("Board of Patent Appeals and Interferences Docketing Notice," Paper 18, issued 5 October 2003).

Other findings of fact follow below.

III. Opinion

28. The examiner relies on the following references (FF 9):

Job et al. (Job)	U.S. Patent 6,107,051	22 August 2000
Badiu et al. (Badiu)	RO 113935 B	29 May 1998

Chachalis et al. ("Smith"), "Seed Soaking Damage in Some Grain Legumes," Journal of New Seeds, Vol. 2, No. 3, pp. 27-36 (2000).

Evans et al. (Evans), "Starting Plants from Seeds," 1/99 HIL-8703, < http://www.ces.ncsu.edu/depts/hort/hil/hil-8703.html > (accessed 21 November 2002).

29. The examiner requested a full text English translation of Badiu on 11 February 2003 (FF 18) after finally rejecting claims 1-3 on 26 November 2002 (FF 9), although

Badiu apparently had been in the examiner's possession since 22 August 2000 (FF 6).

30. The record does not indicate whether or not the examiner provided appellant with the full text English translation of Badiu first supplied to the Board on 1 September 2003 (FF 24).

31. Thus, it does not appear that either the examiner or appellant ever considered the full text English translation of Badiu.

32. It further appears that the examiner relied only on the "Smith" abstract, rather than the full length technical article authored by Demosthenis Chachalis and Mike L. Smith (FF 12-14).

33. Again, the record does not indicate whether or not either the examiner or appellant considered the full length article by Chachalis and Smith.

34. Facially, Evans appears to be a horticultural information leaflet, printed off the internet on 21 November 2002, which appears to have been publicly available in January 1999, based upon a designation "1/99 HIL-8703" beneath its title "Starting Plants from Seeds."

35. As appellant has not challenged the examiner's assertion of a January 1999 publication date and such a date of public availability appears facially reasonable, we accord Evans a public availability date of 31 January 1999. Accordingly, Evans qualifies as prior art under 35 U.S.C. § 102(b).

A. Legal standard

The examiner has the initial burden of establishing a prima facie case of obviousness. In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir.

1993). In rejecting a claim under 35 U.S.C. § 103, the examiner must provide a factual basis to support the obviousness conclusion. In re Freed, 425 F.2d 785, 788, 165 USPQ 570, 572 (CCPA 1970). Based on the objective evidence of record, the examiner is required to make the factual inquiries mandated by Graham v. John Deere Co., 383 U.S. 1, 17, 148 USPQ 459, 469 (1966). The examiner is also required to explain why one having ordinary skill in the art would have been led to modify and/or combine the applied prior art to arrive at the claimed invention. To this end, there must be some teaching, suggestion or inference in the prior art to make the modification and it can not come from appellant's disclosure. Uniroyal, Inc. v. Rudkin-Wiley Corp., 837 F.2d 1044, 1051, 5 USPQ2d 1434, 1438 (Fed. Cir. 1988), cert. denied, 488 U.S. 825 (1988); Ashland Oil, Inc. v. Delta Resins & Refractories, Inc., 776 F.2d 281, 293, 227 USPQ 657, 664 (Fed. Cir. 1985), cert. denied, 475 U.S. 1017 (1986). These showings by the examiner are an essential part of complying with the burden of presenting a prima facie case of obviousness. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Therefore, the examiner should point out where the reference(s) applied under 35 U.S.C. § 103 disclose(s) or suggest(s) all the claim limitations, provide(s) some suggestion or motivation to modify the references to obtain appellant's claimed invention and a reasonable expectation of success in doing so.

B. Discussion

Here, the examiner has relied on two abstracts without reference to the underlying documents, i.e., a full text English translation of Badiu and a full text technical journal article by Chachalis and Smith, where both the abstracts and the

underlying documents are prior art. Citation of an abstract without citation and reliance on the underlying document itself is generally inappropriate where both the abstract and the underlying document are prior art. First, an abstract by its very nature does not reflect the full spectrum of teachings afforded by the underlying reference. In general, an abstract does not contain enough information to enable the subject matter of the underlying reference to be properly evaluated. The main purpose of an abstract is to provide a brief summary which alerts a reader to a possible area of interest, much like a headline in a newspaper. Second, abstracts are often not written by the author of the underlying document and may be erroneous, misleading and/or incomplete. In short, an abstract and its underlying reference are distinct documents.

As set forth in Ex parte Gavin, 62 USPQ2d 1680, 1684 (BPAI 2001),[4]

> [w]hen an examiner cites and relies only on an abstract, the applicant may wish to obtain a copy of the underlying document and submit a copy to the examiner when responding to a rejection relying on an abstract. In the event a reference is in a foreign language, if the applicant does not wish to expend resources to obtain a translation, the applicant may wish to request the examiner to supply a translation. If a translation is not supplied by the examiner, the applicant may wish to consider seeking supervisory relief by way of a petition (37 CFR § 1.181) to have the examiner directed to obtain and supply a translation. In the past, when neither the examiner nor the applicant rely on the underlying article, the board has often expended the resources necessary to obtain a copy of the underlying scientific article, as well as translations thereof. When it did so, however, the burden of examining the application fell on the board in the first instance. Moreover, to the extent that the board relies on parts of a translation not previously provided to an applicant, any affirmance generally has to be a new ground of rejection under 37 CFR § 1.196(b) -- which can result in further prosecution.

[4] See Semiconductor Energy Lab. v. Samsung Elec. Co., 204 F.3d 1368, 54 USPQ2d 1001 (Fed. Cir. 2000) for a discussion of an applicant's obligation with respect to cited foreign language documents.

See also Ex parte Jones, 62 USPQ2d 1206, 1208-09 (BPAI 2001).

Moreover, the Board's position regarding reliance upon abstracts and foreign language documents in support of a rejection as it relates to examination of patents was adopted by the USPTO and incorporated into the February 2003 revision of the eighth edition of the "Manual of Patent Examining Procedure" (MPEP) § 706.02 at pages 700-20 to 700-21[5] (and remains in the current May 2004 revision of the MPEP).

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[5] > **RELIANCE UPON ABSTRACTS AND FOREIGN LANGUAGE DOCUMENTS IN SUPPORT OF A REJECTION**

Prior art uncovered in searching the claimed subject matter of a patent application often includes English language abstracts of underlying documents, such as technical literature or foreign patent documents which may not be in the English language. When an abstract is used to support a rejection, the evidence relied upon is the facts contained in the abstract, not additional facts that may be contained in the underlying full text document. Citation of and reliance upon an abstract without citation of and reliance upon the underlying scientific document is generally inappropriate where both the abstract and the underlying document are prior art. See *Ex parte Jones*, 62 USPQ2d 1206, 1208 (Bd. Pat. App. & Inter. 2001) (unpublished).

To determine whether both the abstract and the underlying document are prior art, a copy of the underlying document must be obtained and analyzed. If the document is in a language other than English and the examiner seeks to rely on that document, a translation must be obtained so that the record is clear as to the precise facts the examiner is relying upon in support of the rejection. The record must also be clear as to whether the examiner is relying upon the abstract or a full text document to support a rejection. The rationale for this is several-fold. It is not uncommon for a full text document to reveal that the document fully anticipates an invention that the abstract renders obvious at best. The converse may also be true, that the full text document will include teachings away from the invention that will preclude an obviousness rejection under 35 U.S.C. 103, when the abstract alone appears to support the rejection. An abstract can have a different effective publication date than the full text document. Because all patentability determinations are fact dependent, obtaining and considering full text documents at the earliest practicable time in the examination process will yield the fullest available set of facts upon which to determine patentability, thereby improving quality and reducing pendency.

When both the abstract and the underlying document qualify as prior art, the underlying document should normally be used to support a rejection. In limited circumstances, it may be appropriate for the examiner to make a rejection in a non-final Office action based in whole or in part on the abstract only without relying on the full text document. In such circumstances, the full text document and a translation (if not in English) may be supplied in the next Office action. Whether the next Office action may be made final is governed by MPEP § 706.07(a).

To summarize, the Board's position on relying on abstracts and foreign language documents in rejecting claims was clearly set forth before any Office action ever issued in this application (see Gavin and Jones; FF 2). There is no evidence of record that appellant requested the examiner to supply a full-text English language translation of Badiu or the full text Chachalis and Smith article underlying the "Smith" abstract being relied upon by the examiner as prior art. There is no explanation why the examiner forwarded the application to the Board in August 2003 without a full-text English language translation of Badiu or the full text article by Chachalis and Smith article given USPTO policy as adopted in the February 2003 revision of the MPEP. To decide an appeal under these circumstances is inconsistent with both the Board's position and USPTO patent policy regarding reliance upon abstracts and foreign language documents in support of a rejection. Therefore, the decision of the examiner to reject claim 1 under 35 U.S.C. § 103 over either Badiu and Smith or Job and Smith, to reject claims 2 and 3 under 35 U.S.C. § 103 over Badiu and Evans, and to reject claim 2 over Job and Evans is vacated.

C. Other issues

Upon return of the application to the jurisdiction of the examiner, the examiner should consider the following issues and take appropriate action.

First, the examiner contends that the term "in a dark place" is a relative term that has not been clearly defined in appellant's specification (Answer, sentence bridging pp. 3-4). Yet, none of appellant's claims stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Moreover, the meaning of a term in a claim must be

consistent with its appearance in other claims in the same patent. Fonar Corp. v. Johnson & Johnson, 821 F.2d 627, 632, 3 USPQ2d 1109, 1113 (Fed. Cir. 1987).

Here, claim 1 recites leaving the seed in a highly watery condition "in a dark place," while claim 2/1 requires the seed to be dried "in insufficient light to cause the seed to germinate." Claim 3/2/1 further requires drying "in a dark place." Therefore, the examiner should interpret for the record, including citation to the record supporting any asserted interpretation, the claim terms (a) "in a dark place" as recited in claims 1 and 3 and (b) "immediately" as recited in claim 1.

Additionally, the claimed invention requires seeds "of a plant which tends to suffer from defective germination or rosette formation during growth thereof." Neither the examiner nor appellant has established on the record whether all or only some, and if so which, plants tend to suffer from defective germination or rosette formation during growth thereof. Therefore, this point should be clarified on the record as well.

Second, we are enclosing copies of (a) the full text English translation of Badiu supplied by the examiner (Exhibit 1004), (b) the full text technical article by Chachalis and "Smith" (Exhibit 1005) and (c) the full text technical article by Corbineau[6] (Exhibit 1006) for initial consideration by the examiner and appellant. Such consideration may supply additional relevant evidence on issues of anticipation and obviousness, as well as provide accurate characterizations of their respective teachings and may eliminate the need for a further appeal.

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[6] The examiner rejected original claim 1 as anticipated by the abstract of Corbineau in the first Office action on the merits (FF 3 and 4). As pointed out by appellant (FF 8), the language of the Corbineau abstract is inconclusive. Therefore, we have obtained a copy of the full length Corbineau article for the examiner's and appellant's consideration.

Third, it appears that the examiner intended to reject claims 2 and 3 over Badiu, Smith and Evans, not just over Badiu and Evans, in referring to "Badiu et al as modified" (Paper 6, p. 3).

Four, Job expressly cites "Coolbear, P., Newell, A.J. & Bryant, J.A. (1987) Ann. Appl. Biol. 110, 185-194 ("Coolbear") as the source of the described pre-germination treatment by hydroconditioning at low temperature (c. 3, ll. 32-34 and 42; c. 4, l. 14; c. 16, ll. 10-11). Therefore, consideration of Coolbear, if available, may supply additional relevant evidence and a more detailed description of pre-germination treatment by hydroconditioning at low temperature.

We take no position on the ultimate merits of any rejection under 35 U.S.C. §§ 102 and/or 103 based upon the full text English translation of Badiu, the full text Chachalis and "Smith" article, a reconsideration of Job or any additional prior art which the examiner and appellant have made or wish to make of record, particularly in light of the recommended considerations upon remand.

VI. Order

Upon consideration of the record, and for the reasons given, it is

ORDERED that the decision of the examiner rejecting (1) claim 1 under 35 U.S.C. § 103(a) over Badiu's abstract in view of Smith's abstract, (2) claims 2 and 3 under 35 U.S.C. § 103(a) over Badiu's abstract, evidently in view of Smith's abstract, and further in view of Evans, (3) claim 1 under 35 U.S.C. § 103(a) over Job in view of Smith's abstract, and (4) claim 2 under 35 U.S.C. § 103(a) over Job in view of Evan is **vacated**.

FURTHER ORDERED that the application is **remanded** to the examiner for action not inconsistent with the views expressed in this opinion.

FURTHER ORDERED that no time period for taking any subsequent action in this appeal may be extended under 37 CFR § 1.136(a).

VACATED AND REMANDED

McK

FRED E. McKELVEY, Senior
Administrative Patent Judge

RICHARD TORCZON
Administrative Patent Judge

Carol A. Spiegel

CAROL A. SPIEGEL
Administrative Patent Judge

BOARD OF PATENT
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13/3,K/30 (Item 13 from file: 50)
DIALOG(R)File 50:CAB Abstracts

02678662 CAB Accession Number: 930320520
Effects of priming on the germination of Valerianella olitoria seeds in relation with temperature and oxygen.
Corbineau, F.; Come, D.
U.A. 1180, CNRS, Universite Pierre et Marie Curie, 75252 Paris Cedex 05, France.
Acta Horticulturae (No. 267): p.191-197
Publication Year: 1990
ISSN: 0567-7572 --
ISBN: 90-6605-134-5
Language: English
Document Type: Conference paper; Journal article

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... also assayed at 20 deg under different O2 concentrations (3, 5, 10, 15 and 20%). *Seeds* were primed by soaking in aerated, deionized *water* for 40 h at 20 deg C in *darkness* and either germinated directly or after redrying to 6-8% moisture content. *Seeds* were *stored* at 20 deg and 55% RH for up to 6 weeks. The optimum temperature for...

EX1001

Journal of

New Seeds™

Volume 2
Number 3
2000

CONTENTS

Soybean (*Glycine max* [L.] Me ops grown in the United States. From a crop wit it the turn of this century, soybeans have made r ure, claiming the stature of the number two U.S. it source of edible vegetable oil and protein thro multitude of food and industrial applications. E urce of protein for livestock and humans, the nu ins is not optimal. Some of the problems associated de (1) presence of anti-nutritional factors such as trypsin inhibitor, (2) undesirable beany flavor, (3) elicitation of allergic reactions in susceptible individuals, (4) poor digestibility of soybean proteins, and (5) deficiency in sulfur-containing amino acids. As a consequence, concerted efforts are underway to improve the overall nutritive value of soybean proteins by both classical plant breeding and molecular biological approaches. This review article summarizes the current knowledge on the biochemistry and molecular biology of soybean seed storage proteins. Recent advances in the genetic improvement of amino acid composition of seed storage proteins are highlighted. The review also includes some recent achievements in modifying soybean seed composition.

Ex 1002

KEYWORDS. □-conglycinin, glycinin, nutritional quality, storage proteins

(1)

Seeds of soybeans (*Glycine max*), peas (*Pisum sativum*), faba beans (*Vicia faba*) and dwarf beans (*Phaseolus vulgaris*) were used. The effects of soak water whether untreated, air-enriched or CO_2-enriched on germination, membrane integrity and the vital staining with tetrazolium chloride of embryo tissues were assessed. Imbibition damage, expressed as low percentage of embryo tissue stained with tetrazolium chloride, was observed when seeds soaked in water. In soybeans and faba beans, imbibition damage was enhanced by the CO_2-enriched soaking treatment whereas in peas imbibition damage was greater after soaking in air-enriched soak water. Most of the soak-damaged seeds could not sustain normal growth as indicated by the reduced percentage of normal seedlings or low embryonic axis growth recorded. Where soaking reduced germination, seed drying after soaking resulted in increased germination capacity, possibly because membrane integrity and embryo viability were restored upon drying.

SAC 1958

KEYWORDS. Germination, leakage, legume seeds, imbibition damage, vital staining

Greenhouse and growth chamber experiments were conducted to examine glyphosate [isopropylamine salt of *N*-(phosphonomethyl) glycine] effects on growth, chlorophyll content, nodulation, and nodule leghemoglobin content of glyphosate-resistant and susceptible soybean (*Glycine max* [L.] Merr.) varieties. In susceptible soybean, a single application of 0.28 kg/ha reduced chlorophyll content (49%), and shoot and root dry weight (50 and 57%, respectively) at 2 wk after treatment. In glyphosate-resistant soybean, there were no significant effects on these parameters by single application up to 1.12 kg/ha, but 2.24 kg/ha reduced shoot and root dry weight by 25 to 30%. Application of glyphosate 1.12 kg/ha, followed by sequential applications at 0.56 or 1.12 kg/ha, did not affect plant growth and chlorophyll content, but application of 2.24 kg/ha followed by sequential application of 2.24 kg/ha reduced root growth. In glyphosate-resistant soybean, an application of 1.12 kg/ha 3 wk after planting did not affect nodule number or mass, but 2.24 kg/ha reduced these parameters by 30 and 39%, respectively, compared to untreated. Leghemoglobin content of nodules was reduced (6 to 18%) by both glyphosate rates, but effects were inconsistent with rate. At post-treatment temperatures of 18/13°C (day/night), glyphosate at 1.12 kg/ha or 2.24 kg/ha did not affect chlorophyll and growth of glyphosate-resistant soybean. However, at 25/20 and 32/27°C (day/night), glyphosate at 2.24 kg/ha reduced both chlorophyll content and growth of glyphosate-resistant soybean. Overall, treatment of glyphosate-resistant soy-

(2)

bean with glyphosate at 1.12 had little or no effect on chlorophyll content and dry weight of shoots and roots in five of five trials. But treatment of glyphosate at 2.24 kg/ha reduced these parameters in three of five trials, suggesting potential for soybean injury at higher rates. Results showed subtle reductions of nodulation in glyphosate-resistant soybean using label rates of glyphosate, but these effects may be of minimal consequence due to the potential of soybean to compensate after short durations of stress.

KEYWORDS. Leghemoglobin, glyphosate, herbicide-resistant crops, nodule, rhizobium, transgenic crops

A novel and general strategy for the management of plant genes for pest or pathogen resistance is presented. The strategy utilizes F_2 hybrids produced from two parents similar in agronomic and quality traits but which differ in their plant genes for resistance to a pest or pathogen. The grower would plant F_2 hybrid seed that should allow for the expression of hybrid vigor for agronomic and quality traits while presenting a diverse population of plants to the pest or pathogen in which individual plants differ for their pest resistance genotype. This strategy will be suitable especially in self-pollinated crops like cotton (*Gossypium* spp.) where there is no significant effect of inbreeding depression. This simulates nature where the pest or pathogen is exposed to a population of host plants that differ in resistance genes. This strategy should delay or minimize the selection of a dominant pest or pathogen biotype that can damage and reproduce on the resistant plants resulting in the loss of the usefulness of the resistant genes. Careful selection of individual genes should allow this strategy to utilize the advantages of strong vertical resistance genes while maintaining the sustainability advantages of horizontal resistance. This strategy also provides for a refuge of a small percentage of the plants in an F_2 population which do not carry any resistance genes, thus providing an additional benefit in resistance management against genetically engineered pest resistant crops. However, this method will not be suitable for crops with significant effect of inbreeding depression.

KEYWORDS. Pest resistance, F_2 hybrid, *Bt* gene, resistance management, cotton, multiline, genetic diversity

SEED TESTING AND REGULATION

REPORT

What Is Seed Vigour?

67

Albie van de Venter

Probably no other component of seed quality has been the subject of as much controversy and misunderstanding as seed vigour. Although the complex underlying mechanisms of seed vigour have not been fully described, much progress has been made in our understanding of the phenomenon and its practical consequences. This first of two articles on seed vigour and seed vigour testing summarizes our current understanding of this seed property.

KEYWORDS. Seed quality, seed vigour, seed vigour testing

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U. S. Serial No. : 10/007,186

Requester's Name: Andrea Valenti
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Office Location: CPK5 3U11
Art Unit/Org. : 3643
Group Director:
Is this for Board of Patent Appeals? Yes
Date of Request: 2/11/03
Date Needed By: 3/11/03
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1. ✓ Patent
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   Language Romanian
   Country Code RO
   Publication Date 01/19/96
   No. of Pages 3 *(filled by STIC)*

2. ____ Article
   Author
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3. ____ Other
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EX1003

PTO 01-[2003-1828]

German Patent
Document No.113935 B

Title

METODĂ PENTRU CREŞTEREA GERMINAŢIEI ŞI REDUCEREA DORMANŢEI SEMINŢELOR DE SFECLĂ DE ZAHĂR

Authors

Aurel Florentin BADIU (Bucharest, Romania)
Aurica BAIA (Fundulea, Romania)

Ex1004

UNITED STATES PATENT A
Washington, D.C.)3

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Country	:	ROMANIA
Document No.	:	RO 113935 B
Document Type	:	PATENT-RELATED ORIGINAL
Language	:	ROMANIAN
Inventor(s)	:	Aurel Florentin BADIU, Aurica BAIA
Applicant	:	RESEARCH INSTITUTE FOR THE CULTIVATION AND PRODUCTION OF SUGARBEET AND SWEET SUBSTANCES, FUNDULEA, ROMANIA
IPC	:	A 01 N 33/12
Application Date	:	JANUARY 19, 1996
Publication Date	:	MAY 29, 1998
Foreign Language Title	:	METODĂ PENTRU CREŞTEREA GERMINAŢIEI ŞI REDUCEREA DORMANŢEI SEMINŢELOR DE SFECLĂ DE ZAHĂR
English Title	:	METHOD OF IMPROVING GERMINATION PERFORMANCE AND REDUCING DORMANCY

FOR SUGAR BEET SEEDS

Method Of Improving Germination Performance /1[1]

And Reducing Dormancy For Sugar Beet Seeds

Specification: The invention named herein refers to a treatment method of improving germination performance and of reducing dormancy for monogerm sugar beet seeds, a method used by the experiment stations when processing the seeds before sowing. The sugar beet seeds are steeped for 6-8 h in a dish containing running tap water at 15-18°C (a temperature maintained by the appropriate adjustment of the water cock), and then they are dried in hot air (30-35°C) to 14% moisture. If the seeds are to be pelleted, the drying could be performed by introducing the seeds into the pelleting equipment.

The invention named herein refers to a treatment method of improving germination performance and reducing dormancy for monogerm sugar beet seeds, a method used by the experiment stations when processing the seeds before sowing.

As dormancy represents an inherent characteristic of the seeds, it cannot be dealt with mechanically. To date, as no methods have been available to mechanically or physically remove the

[1] Numbers in the margin indicate pagination in the foreign text.

dormant seeds from the total amount of the viable seeds, seedlots with 15-20% content of dormant seeds are destroyed.

However, one method would be to treat these seeds with hydrogen peroxide which stimulates germination and reduce dormancy of the monogerm sugar beet fruits (M. Coumans - "Etudes des obstacles a le germination chez le bettarave sucriere" - Institut Van Beneden 1978).

This method showed a few shortcomings, one of them being the fact that hydrogen peroxide is a highly oxidant chemical which can be poisonous for mucous membranes or tissues. A higher concentration of hydrogen peroxide triggers the inhibition of germination.

The oxygen peroxide is highly corrosive, so the equipment needs protection against corrosion (inox).

Another method would be the treatment of the seeds using phytohormones which can activate the process of germination (B. de Vergnes - "L'activation des semences de betterave sucriere", 1995), but this method involves high costs. The growers are also aware of another treatment method in which sugar beet seeds are soaked in hot water at 25°C (R. Saboljevic - "Germination variability of sugar beet seeds in relation to different temperatures and the leaching procedures", 1995), but this

method shows a shortcoming: the water temperature must be constantly maintained at 25°C, throughout the process.

The technical issue which is dealt with successfully by this here method is the reducing of the dormant glomerules content using an efficient, low-cost, non-pollutant, non-toxic and easy-to-perform treatment.

The procedure used for this method eliminates all the shortcomings of all the other available methods and has this new procedure of soaking the seeds in running tap water (flow speeds over the range 0.25-0.5 m/s) at 15-18°C for 6-8 h, then drying them to 14% moisture using hot air (max. 30-35°C) or pelleting equipment.

This method makes the following improvements:

- increases the quality of the monogerm sugar beet seeds;
- it is simple, low-cost, non-toxic and non-pollutant;
- the effect of the treatment is present for a period of about 120 days after its completion, a fact that reduces dormancy with 50-80% and increases germination with 5-10% depending on the concentration of the dormant glomerules for field and laboratory conditions.

The procedure used for this method was as follows.

The monogerm sugar beet seeds to be treated have below 80% germination and 8-10% content of dormant seeds. They are soaked

for 6-8 h in running tap water at 15-18°C, values that varies with the concentration of the dormant glomerules and seedlot characteristics.

The seeds are steeped in a dish with running tap water at a flow speed of 0.25-0.5 m/s, a range that is maintained by the appropriate adjustment of the water cock. When the steep is completed, the seeds are dried at 30-35°C to 14% moisture or they are merely pelleted. The effect of the treatment is present for 90-120 days after its completion. /2

Claim

The invention named herein refers to a treatment method of improving germination performance and reducing dormancy for monogerm sugar beet seeds, and claims that the seeds are steeped for 6-8 h in a dish containing running tap water (flow speed at 0.25-0.5 m/s) at 15-18°C (a temperature maintained by the appropriate adjustment of the water cock), and then they are dried in hot air (30-35°C) to 14% moisture, or merely pelleted.

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M. Coumans, *Etude des obstacles a la germination chez la betterave sucriere*, Institut Van Beneden, 1978.

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(54) METODĂ PENTRU CREŞTEREA GERMINAŢIEI ŞI REDUCEREA DORMANŢEI SEMINŢELOR DE SFECLĂ DE ZAHĂR

(57) **Rezumat:** Invenţia se referă la o metodă de tratare a seminţelor de sfeclă de zahăr monogerme, în vederea creşterii germinaţiei şi reducerii dormanţei acestora, metodă care se utilizează în staţiile specializate, pentru condiţionarea seminţelor înaintea însămânţării lor. Seminţele de sfeclă de zahăr se imersează timp de 6...8 h într-un bazin (cuvă) cu un curent continuu de apă potabilă curentă, cu temperatura de 15...18°C, cu debit de 0,25...0,50 m/s, realizat prin reglarea corespunzătoare a robinetului de admisie a apei, după care acestea se usucă în curent de aer cald, (30...35°C), până când ajung la umiditatea de 14%. Dacă seminţele se folosesc pentru drajare, uscarea seminetelor se poate face prin introducerea lor în fluxul de drajare/încrustare.

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Invenția se referă la o metodă pentru creșterea germinației și reducerea dormanței semințelor din sfeclă de zahăr, utilizată în stațiile specializate pentru condiționarea semințelor înaintea însămânțării lor.

Semințele dorminde nu se pot îndepărta prin mijloace mecanice, dormanța fiind o caracteristică specifică intrinsecă. Loturile în care ponderea acestor semințe este mai mare de 15...20% se distrug, până în prezent neexistând nici o metodă fizico-mecanică care să poată elimina aceste semințe din masa semințelor potențial germinabile.

Este cunoscută o astfel de metodă, prin care semințele de sfeclă de zahăr sunt tratate cu apă oxigenată, având în vedere acțiunea apei oxigenate asupra germinației și asupra dormanței la fructele monogerme de sfeclă de zahăr (M. Coumans - "Etude des obstacles a la germination chez la betterave sucrière" Institut Van Beneden 1978).

Această metodă prezintă o serie de dezavantaje, printre care acela că apa oxigenată care se utilizează este un produs chimic puternic oxidant, toxic pentru mucoase și tegumente. La doze ridicate de apă oxigenată, se realizează o inhibare a germinației.

Apa oxigenată este puternic corosivă, instalațiile de tratare necesitând protecții speciale (inox).

Mai este cunoscută tratarea semințelor de sfeclă de zahăr cu fitohormoni, care să inițializeze procesele de germinare (B de Vergnes -" L'activation des semences de betterave sucrière", 1995), tratare care se realizează însă cu costuri ridicate. Se cunoaște, de asemenea, tratamentul care se aplică semințelor de sfeclă de zahăr cu apă caldă la 25°C (R.Saboljevic -"Germination variatibility of sugar beet seeds in relation to defferent temperatures and the leaching procedures", 1995), dar care prezintă dezavantajul menținerii constante a apei la temperatura de 25°C, pe tot parcursul tratamentului.

Problema tehnică pe care o rezolvă invenția constă în reducerea ponderei

glomerulelor dorminde la sfecla de zahăr, prin aplicarea unui tratament eficient, ieftin, netoxic sau nepoluant și ușor de aplicat.

Metoda, conform invenției elimină dezavantajele metodelor cunoscute și se deosebește de acestea prin faptul că semințele de sfeclă de zahăr monogerme se tratează cu un flux continuu de apă potabilă curentă, cu temperatura de 15...18°C, cu debit de 0,25... 0,50 m/s, timp de 6...8 h, după care se usucă în curent de aer cald, la temperaturi maxime de 30...35°C, până când acestea ajung la umiditatea de 14% sau se introduc în fluxul de drajare/încrustare în care se va realiza uscarea lor.

Prin aplicarea invenției se obțin următoarele avantaje:

- crește calitatea culturală a semințelor de sfeclă de zahăr monogerme, în special la loturile semincere;
- este simplă, ieftină, netoxică și nepoluantă;
- efectul tratamentului se menține peste 120 zile și elimină dormanța în procent de 50...80% și crește potențialitatea germinației în procent de 5... 10%, proporțional cu ponderea glomerulelor dorminde în condiții de laborator și câmp.

Se prezintă în continuare un exemplu de realizare a invenției.

Metoda, conform invenției constă în tratarea semințelor de sfeclă de zahăr monogerme, cu germinație sub 80% și conținut de semințe dorminde de peste 8...10%, cu un curent continuu de apă potabilă curentă cu temperatura de 15...18°C, timp de 6...8 h, în funcție de ponderea glomerulelor dorminde și specificitatea lotului de semințe tratat.

Semințele se imersează într-un bazin (cuvă), asigurându-se un flux continuu de apă potabilă curentă, cu viteza de curgere de 0,25...0,50 m/s, prin reglarea corespunzătoare a robinetului de admisie a apei. După parcurgerea timpului, semințele de sfeclă de zahăr se usucă la temperatura de max.30...35°C, până când acestea ajung la umiditatea de 14%, sau se introduc în fluxul de dra

jare/încrustare în care se va realiza uscarea lor. Efectul tratamentului se menține între 90 și 120 zile de la efectuarea lui.

Revendicare

Metodă de tratare a semințelor de sfeclă de zahăr monogerme în vederea creșterii germinației și reducerii dormanței acestora, **caracterizat prin aceea că** semințele de sfeclă de zahăr se imersează timp de 6...8 h într-un bazin (cuvă) cu un curent continuu de apă potabilă curentă, cu temperatura de 15...18°C, cu viteza de 0,25...0,50 m/s, realizată prin reglarea corespunzătoare a robinetului de admisie a apei, după care acestea se usucă în curent de aer cald (30...35°C), până când ajung la umiditatea de 14%, sau se introduc în fluxul de drajare/încrustare în care se va realiza uscarea lor.

Președintele comisiei de examinare: **dr. ing. Paraschiv Adriana**
Examinator: **ing. Popescu Mădălina**

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TITLE: Method to improve germination rate and reduce sugar-beet seed dormancy
- by immersion in running tepid potable water and drying in warm air

INVENTOR: BADIU, A F; BAIA, A

PATENT-ASSIGNEE: INST CERC PROD CULTURA IND SFECLEI ZAHAR [CULTN]

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BASIC-ABSTRACT: Germination of sugar-beet seeds is increased and their dormancy reduced by the following treatment:(a) the seeds are immersed for 6-8 hrs in a vat containing a continuous potable water current of 0.25-0.50 m/s inlet flow velocity and of 15-18 deg. C; (b) the seeds are then dried in a current of warm air at 30-35 deg. C until their moisture content is at 14%. Drying may be combined with an encapsulation dragee-forming process.

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TITLE-TERMS:
METHOD IMPROVE GERMINATE RATE REDUCE SUGAR BEET SEED DORMANT IMMERSE RUN POTABLE WATER DRY WARM AIR

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Seed Soaking Damage in Some Grain Legumes

Demosthenis Chachalis
Mike L. Smith

ABSTRACT. Seeds of soybeans (*Glycine max*), peas (*Pisum sativum*), faba beans (*Vicia faba*) and dwarf beans (*Phaseolus vulgaris*) were used. The effects of soak water whether untreated, air-enriched or CO_2-enriched on germination, membrane integrity and the vital staining with tetrazolium chloride of embryo tissues were assessed. Imbibition damage, expressed as low percentage of embryo tissue stained with tetrazolium chloride, was observed when seeds soaked in water. In soybeans and faba beans, imbibition damage was enhanced by the CO_2-enriched soaking treatment whereas in peas imbibition damage was greater after soaking in air-enriched soak water. Most of the soak-damaged seeds could not sustain normal growth as indicated by the reduced percentage of normal seedlings or low embryonic axis growth recorded. Where soaking reduced germination, seed drying after soaking resulted in increased germination capacity, possibly because membrane integrity and embryo viability were restored upon drying. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

KEYWORDS. Germination, leakage, légume seeds, imbibition damage, vital staining

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INTRODUCTION

Legume seeds frequently experience waterlogged conditions due to rainfall immediately after planting that could result in poor crop stands (Troedson et al., 1983; Hwang and Sung, 1991). Powell and Matthews (1978) firstly described imbibition damage as the poor embryo staining with tetrazolium chloride coupled with high electrolyte leakage that results from rapid water uptake during imbibition. Powell et al. (1984) proposed that imbibition damage was a common situation in all grain legumes, being observed in soybeans (Oliveira et al. 1984), dwarf beans (Powell et al. 1986), chickpeas (Knights and Mailer, 1989; Legesse and Powell, 1992), long beans (Abdullah et al. 1992), and faba beans (Rowland 1981; Kantar et al. 1996).

Some earlier reports have suggested that the fundamental cause of germination failure in *P. vulgaris* seeds was the loss of essential cell constituents during soaking in water (Barton, 1950; Barton and McNab, 1956).

An additional problem in waterlogged soil is anoxia (a deficiency of oxygen), and its effects on seeds are numerous and complex. Excessive soil moisture displaces air, slows oxygen diffusion through soil, and increase levels of CO_2 (Norton 1986). Orphanos and Heydecker (1968) ascribed low germination of *P. vulgaris* seeds to a deficient oxygen supply, at a post-soaking period that coincided with the critical early stage of germination. Reduction in seedling emergence under flooding has been also attributed to toxic effects of either ethanol self-poisoning (McManmon and Crawford, 1971), or volatile aldehydes particularly in low vigour soybean and pea seeds (Harman et al., 1981).

Seeds sensitive to soaking damage could either completely run out of available energy (Norton, 1986) or remaining sucrose level was not sufficient to support germination (Pretorious and Small, 1993). It is therefore important to measure the growth capacity of axes from soak-damaged seeds when grown with or without readily available energy.

There has been little information on how imbibition damage (revealed by high leakage and low embryo staining) is affected by the gaseous composition of soak water using seeds of different grain legumes species. The aim of this study, using four different legume species, was to determine how enrichment of the seed soak water with carbon dioxide or air affected germination, membrane integrity and embryo viability.

MATERIALS AND METHODS

Studies were conducted on high quality seeds of soybeans (*G. max* L. cv. Sapporo) obtained from Rustica Semences, peas (*P. sativum* L. cv. Onward), dwarf beans (*P. vulgaris* L. cv. Masterpiece), and faba beans (*V. faba* L. cv. Maris Bead) obtained from Nickerson Seeds Ltd., UK.

Batches of seeds were imbibed in water subject to the following treatments: no treatment (control), water (W), air enriched water (A-W) and CO_2 enriched water (CO_2-W). The gas enriched water was prepared by bubbling either respective gas through distilled water for 10 minutes in glass jars (Schott, capacity 1.5 L). Following addition of seeds, these were sealed with rubber septa. The pH of the water was measured twice, immediately after the 10 minutes of gas bubbling and at the end of a 24 h soaking period. The pH values of the soaking treatments were as following: W, 5.6; A-W: 5.3 (10 min), 5.7 (24 h); CO_2-W: 4.3 (10 min), 4.9 (24 h).

Seeds were weighed, surface-sterilized in 4% w/v sodium hypochloride for 15 seconds and then washed in distilled water. The seeds were then soaked for 24 h at 25°C in either 250 ml distilled water or gas-enriched water. After addition of the seeds to the jars, the jars were sealed and placed in an incubator (Stuart SI60) until the end of the soaking treatment. Four replicates of 20 seeds from each treatment were incubated at 25°C for 5 days in 90 mm glass Petri dishes on a single sheet of filter paper (Whatman, grade 181) moistened with 4 ml water. Control, unsoaked seeds (i.e., set to germinate as dry seeds) had further 4 ml-distilled water added after the first 12 h incubation. Subsequently, 1 ml of distilled water was added to each treatment every 24 h. Radicle protrusion through the testa was taken as a sign of germination. Seeds were scored for germination each day for 5 d. To study the effect of different soaking treatments on the percentage of normal seedlings produced, seeds were set to germinate after the soaking treatments using the standard rolled paper towel test (ISTA, 1985) with 4 replicates of 25 seeds per treatment. Classification of seedlings as normal was made in accordance with the ISTA regulations (Bekendam & Grob, 1979). The effect of seed drying after soaking on the percentage germination and percentage of normal seedlings was examined by placing seeds under ambient conditions ($\approx$20°C) for 4 d. Subsequently, seeds were air-dried inside the incubator for 4 d at 30°C

until seed moisture contents reached 7-9% f.wt. (oven drying method). Germinability and numbers of normal seedlings were performed and assessed as described above.

The water uptake by seeds during imbibition was determined for 4 replicates of 25 seeds for each species and treatment. Seeds were soaked for 24 h, then blotted dry, and re-weighed with water uptake expressed the percentage weight increase. At the end of the 24 h soaking period, the conductivity of the soak water was also measured and expressed per gram of the fresh weight of the seeds prior to soaking ($\mu Sg^{-1}cm^{-1}$).

The viability of embryo tissues was determined after 24 h soaking by placing seeds from each treatment in a 1% (w/v) solution of 2,3,5 triphenyl tetrazolium chloride (TTC) for 24 h in the dark. The control seeds were soaked n 30% w/v PEG for 24 h prior to staining with TTC. This allows full imbibition to occur, but at no point do the seeds imbibe rapidly (Powell et al. 1986). The numbers of seeds with fully stained cotyledons or embryonic axes was recorded. Four replications of 25 seeds each were assessed.

Growth of the embryonic axes following each of the soak water treatments (from randomly selected seeds) was assessed following separation of the axes from the cotyledons immediately after the 24 h treatments. The excised axes were then placed for growth in Petri dishes on a single sheet of filter paper (Whatman, grade 181) moistened with 4 ml of either water or 1% sucrose solution. Subsequently, the axes were covered with another single sheet of filter paper. Daily, 1 ml of either water or 1% sucrose solution was added. Excised axes from unsoaked dry seeds were used as the control. The embryonic axes were oriented with the abaxial surface in contact with the paper and the radicle tip slanting down. Initial fresh weight and final fresh and dry weights after 7 d incubation at 25°C were determined. Measurements were made on ten excised embryonic axes per Petri dish replicated three times per experiment with each experiment performed at least twice.

RESULTS

Soaking treatments had minimal effect on germination of soybean and faba bean seeds (Table 1). Dwarf bean seeds were more affected by the different soaking treatments that reduced from 98% (control) to

TABLE 1. Percentage germination, percentage normal seedlings of seeds placed for germination either immediately after the soaking treatments or dried before (+D); control (no treatment), W (water soaked), A-W (air-enriched water soaked), CO_2-W (CO_2-enriched water soaked).

	germination (%)				normal seedlings (%)			
	soybeans	peas	faba beans	dwarf beans	soybeans	peas	faba beans	dwarf beans
control	96	96	99	98	92	90	89	90
W	95	95	99	78	87	86	83	67
A-W	95	52	93	53	84	58	79	64
CO_2-W	92	95	97	77	62	79	65	86
control + D	93	96	95	98	91	91	91	89
W + D	88.	93	91	91	89	89	88	86
A-W + D	87	91	88	92	86	86	84	91
CO_2-W + D	86	92	88	93	85	84	87	88
s.e.m. (n = 4)	*2*	*3*	*4*	*3*	*2*	*4*	*3*	*4*

78 and 77% (W and CO_2-W soaking treatments, respectively) and 53% (A-W treatment) (Table 1). The A-W treatment was the most detrimental to germination in peas and dwarf beans. Where germination was clearly reduced by soaking (e.g., peas and dwarf beans), there was an increase in germination if seeds dried after soaking. However, where there was no effect of soaking (e.g., soybean and faba bean), a subsequent drying period reduced germination slightly. Numbers of normal seedlings decreased after all soaking treatments, with a greater decrease where soaking had caused a fall in the total germination (Table 1). In this case drying after soaking resulted in an increase in the percentage of normal seedlings only after CO_2-W treatment on soybean and faba bean, A-W in peas and after W and A-W in dwarf beans. Although, the CO_2-W treatment had little effect on germination of soybeans, peas, and faba beans, percentage of normal seedlings was affected.

Water uptake after 24 h of soaking in soybean, faba bean, and dwarf bean seeds was similar regardless of the soaking treatment (Table 2). In peas, however, A-W soaked seeds absorbed more water than the other treatments (Table 2).

Conductivity was the highest in CO_2-W treatments in soybeans, faba beans, and dwarf beans (Table 2). In peas, the highest conductivity was measured in A-W soaked seeds (Table 2). Imbibition damage

revealed from the conductivity test was clearly associated with low germination and numbers of normal seedlings (A-W treatment in peas) or low numbers of normal seedlings (CO_2-W treatment in soybeans and faba beans). However, in dwarf beans there was no correlation between high leakage (CO_2-W treatment) and low germination (A-W treatment) or numbers of normal seedlings (W and A-W treatment).

The amount of embryo tissues stained with TTC was reduced by the W treatment in all but dwarf bean seeds (Table 3). Soybeans and faba beans showed a greater reduction in staining in the cotyledons than in the axes indicating their greater sensitivity to treatment, with the greatest reduction in the CO_2-W treatment in both cotyledons and axes.

TABLE 2. Percentage weight increase and electrical conductivity of soybean, peas, faba bean and dwarf bean seeds subjected to different soaking treatments; W (water soaked), A-W (air-enriched water soaked), CO_2-W (CO_2-enriched water soaked).

	water uptake (%)				conductivity ($\mu S g^{-1} cm^{-1}$)			
	soybeans	peas	faba beans	dwarf beans	soybeans	peas	faba beans	dwarf beans
W	110	108	96	103	25	25	29	24
A-W	113	162	99	102	26	64	37	28
CO_2-W	114	111	93	101	37	32	47	34
s.e.m. (n = 4)	2	1	2	2	1	2	2	1

TABLE 3. The percentage of cotyledons or embryonic axes fully stained with tetrazolium chloride; control (no treatment), W (water soaked), A-W (air-enriched water soaked), CO_2-W (CO_2-enriched water soaked).

	percentage of seeds fully stained with TTC (%)							
	soybeans		peas		faba beans		dwarf beans	
	cotyledons	axes	cotyledons	axes	cotyledons	axes	cotyledons	axes
control	94	95	93	94	95	97	97	96
W	25	85	18	19	22	63	96	94
A-W	8	22	4	0	5	17	97	96
CO_2-W	0	4	22	17	2	0	95	94*
s.e.m. (n = 4)	4	3	2	3	2	2	3	2

*: radicle tip unstained

However, in peas, the lowest staining was observed after the A-W treatment. The poor staining of the embryonic axes for soybean, pea, and faba bean (0-22%) in the A-W and CO_2-W treatments was associated with either low germination (52% in peas) or low numbers of normal seedlings (62 and 65% in soybeans and faba beans, respectively).

In most cases, the various soak treatments had a negative effect on the axis fresh weight regardless of growing media (Table 4). After the CO_2-W treatment, the low growth of embryonic axes on NS media for soybean and faba bean (23 and 14 mg, respectively) was clearly associated with high leakage (37 and 47 $\mu Sg^{-1}cm^{-1}$, respectively) and low staining (4 and 0%, respectively). In peas, the low growth of embryonic axes (14 mg) on NS media after the A-W treatment was associated with high leakage (64 $\mu Sg^{-1}cm^{-1}$) and low staining (0%). For soybean, pea and faba bean, growing axes with 1% sucrose restored axis growth to similar levels observed for untreated control seeds (Table 4). For dwarf beans, however, no improvement in the axis growth on the sucrose media from A-W soaked seeds was measured (Table 4).

DISCUSSION

Imbibition damage (amounts of embryo tissues stained with TTC) was observed when seeds soaked in water due possibly to rapid water

TABLE 4. Axis growth from soybean, peas, faba bean and dwarf bean seeds in either non-sucrose filter paper (NS-media) or sucrose added filter paper (S-media) that were subjected to different soaking treatments; control (no treatment), W (water soaked), A-W (air-enriched water soaked), CO_2-W (CO_2-enriched water soaked).

	axis fresh weight (mg)							
	soybeans		peas		faba beans		dwarf beans	
	NS-media	S-media	NS-media	S-media	NS-media	S-media	NS-media	S-media
control	49	51	25	29	20	25	38	45
W	39	49	22	28	17	24	33	39
A-W	36	48	14	26	17	23	27	27
CO_2-W	23	47	17	28	14	23	31	38
s.e.m. (n = 4)	*3*	*2*	*2*	*2*	*1*	*2*	*3*	*3*

uptake since staining was typical of that following rapid imbibition. This result is line with the imbibition damage theory introduced by Powell and Matthews (1978). In soybeans and faba beans, imbibition damage was further enhanced by the CO_2-W soaking treatment (Table 3). This may be due to toxic effects of the soaking medium by high ethanol production (McManmon and Crawford, 1971) or high levels of volatile aldehydes produced in the CO_2-W soaking treatment (Harnan et al., 1981). The poor growth capacity of soaked-damaged seeds was reflected in a low numbers of normal seedlings produced and a low axis growth measured. Low axis growth in soaked sensitive seeds indicated partial depletion of substrates that support respiration and the first stage of germination before reserve mobilization starts (Bewley and Black, 1994). When sucrose was added to the water, axis growth capacity was restored to control levels (Table 3).

In peas, low germination and numbers of normal seedlings were recorded in A-W treatment in agreement with a previous report that extra oxygen in the soak water decreased germination and its rate (Norton, 1988). The enhancement of imbibition damage observed when seeds soaked in air-enriched soak water was correlated with the highest leakage of electrolytes, and the least embryo staining. Simon (1984) suggested that lipid peroxidation cause loss of membrane integrity (manifested by high leakage) enables the imbibing seed to achieve higher water content by displacing the natural gas phase.

Soak damaged seeds could also not sustain normal growth as indicated by the low percentage of normal seedling recorded (Table 4). Low axis growth in soak-damaged seeds indicated partial depletion of substrates since growth was restored when sucrose was added to the water (Table 3). Seed drying after soaking increased germination capacity, possibly because membrane integrity and embryo viability were restored upon drying. A similar observation has been previously reported in *P. vulgaris* (Orphanos and Heydecker, 1968), and maize (*Zea mays*) seeds (Martin et al., 1991).

Dwarf bean seeds subjected to either soaking treatment had low germination although little electrolyte leakage and no loss of embryo viability were observed. The reason for such an anomaly is not clear yet. It may be possible that dwarf bean seeds were of the highest vigour level and, therefore, imbibition damage during soaking was not manifested. There have been numerous reports showing a positive correlation of imbibition damage with the vigour level of seeds (Po-

well et al. 1984). In our study, excised axes growth from soak-damaged seeds placed on filter paper with 1% sucrose showed no improvement indicating inability to utilize readily available sucrose in agreement with previous reports (Small et al. 1991; Pretorious and Small, 1993).

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Demosthenis Chachalis & Mike L. Smith, "Seed Soaking Damage in Some Grain Legumes," Journal of New Seeds, Vol. 2, No. 2, pp. 27-36 (2000).

Corbineau, F. & Come, D., "Effects of priming on the germination of Valerianella olitoria seeds in relation with temperature and oxygen," Acta Horticulturae, No. 267, pp. 191-197 (1990).

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EX1006

EFFECTS OF PRIMING ON THE GERMINATION OF VALERIANELLA OLITORIA SEEDS IN RELATION WITH TEMPERATURE AND OXYGEN

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Abstract

Lamb's lettuce (Valerianella olitoria L.) seeds germinate much better at relatively low temperatures (5° to 20°C) than at temperatures above 20°C. However, their sensitivity to high temperatures depends on cultivars and seed batches. The thermal optimum is c. 15°-20°C. Lower temperatures slow down germination, and reveal seed heterogeneity and differences between batches. The germination of lamb's lettuce seeds is also very sensitive to oxygen deprivation.

Presoaking of seeds in water greatly improves the percentage and the rate of germination at suboptimal temperatures or in hypoxia. This stimulatory effect of presoaking persists after redrying and during subsequent storage for at least 2 months. However, presoaked seeds deteriorate faster during storage than untreated seeds.

These results allow a better understanding of the causes of poor field emergence under unfavourable sowing conditions (too low or too high temperatures, or soils with excess of water), and lead to a recommendation of presoaking for improvement of lamb's lettuce seed quality.

1. Introduction

Lamb's lettuce (Valerianella olitoria L.) is an important winter vegetable crop in the western part of France. For improving the production of this crop and for using precision sowing techniques, fast and uniform germination of seeds is required. However, in the field, germination of lamb's lettuce seeds is often unsatisfactory, and results in poor emergence and in heterogeneous populations of plants.

In order to ameliorate the germination, treatment of lamb's lettuce seeds by priming was tried. In this case seeds were presoaked in water. Such a treatment (Heydecker and Coolbear, 1977; Bewley and Black, 1982) results in an improved germination rate of seeds of various species under suboptimal conditions (Heydecker et al., 1975). In the following investigations the effects of temperature and oxygen on the germination of untreated and presoaked seeds will be reported. The behaviour of presoaked seeds after redrying and during storage will be also described.

2. Materials and methods

2.1. Plant material

All experiments were carried out with commercial batches of lamb's lettuce seeds (Jade, Vit and Verte de Cambrai cultivars) received from

the Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL) at Carquefou, near Nantes, France.

2.2. Germination assays

Germinability was tested in samples of 200 seeds placed in 10 cm diameter Petri dishes (50 seeds per dish) on a layer of cotton wool moistened with deionized water. Germination assays were carried out over a range of constant temperatures between 5° and 30°C (± 0.5°C) in darkness.

Studies on the effects of oxygen concentration on germination were performed according to Côme and Tissaoui (1968) and using the device developed by them.

A seed was regarded as germinated when the radicle pierced the seed coat. Germination counts were made every day up to 15 days.

2.3. Priming treatment

For priming, seeds were soaked in aerated deionized water during 40 hours at 20°C in darkness. They were germinated either immediately after soaking or after redrying to 6-8% moisture content (dry weight basis) in air at 20°C and 55% relative humidity.

2.4. Seed storage

Untreated and soaked seeds packed in paper bags were stored at 20°C and 55% relative humidity for various lengths of time, and their germination was tested at 5°, 10° and 20°C.

3. Results

3.1. Sensitivity of untreated seeds to temperature and oxygen

Figure 1 shows the germination of one batch of seeds (Verte de Cambrai cv.) at different temperatures. Seeds germinated much better at temperatures ranging from 5° to 20°C than at higher temperatures. They did not germinate at all at 30°C. At the thermal optimum (15°-20°C) almost all seeds germinated within 7 days. Lower temperatures delayed the onset of germination and reduced germination rate, but did not affect significantly the final germination percentage. At 25°C, the maximal germination percentage decreased markedly. Similar results were obtained with all seed batches tested (table 1). However, the sensitivity of seeds to high temperature (25°C) depended on cultivars and batches. Table 1 also shows that at low temperature (10°C) seed heterogeneity and differences between batches were revealed in most cases. This variability was not clearly observed at 15° or 20°C.

The effect of oxygen concentration on germination at 20°C of seeds of the cultivars Jade and Verte de Cambrai is shown in table 2. Almost all the seeds were able to germinate within 10 or 15 days in any atmospheres containing at least 15% oxygen. The lower the oxygen concentration the slower the germination rate and the lower the final germination percentage. Seeds were practically incapable of germinating in 3% oxygen.

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3.2. Effects of priming

After 40 hour-presoaking at 20°C the seeds were not visibly germinated, but if placed, without redrying, at 5°, 10° and 20°C they germinated much faster and in higher percentage than the untreated seeds, especially at the lowest temperatures (table 3). This stimulatory effect of presoaking was maintained when seeds were redried to their original moisture content (6-8%) before testing their germinability (table 3). Germination of seeds at high temperatures (25° or 30°C) was also markedly improved by presoaking for 40 hours whether followed or not by redrying (not shown). Futhermore, presoaking and redrying rendered the seeds less sensitive to oxygen deprivation (figure 2).

Presoaking of shorter periods than 40 hours was also effective on subsequent germination, however to a lesser extent (not shown). A longer presoaking period induced the germination of some seeds, during the treatment, which were killed or gave rise to abnormal seedlings when redried.

3.3. Effects of storage

Table 4 shows the change in germinability of untreated and 40 hour-presoaked seeds during 26 weeks of storage at 20°C and 55% relative humidity. Germination of untreated seeds at 5°, 10° and 20°C was not affected by length of storage. Presoaked seeds lost their germinability after approximately 2 months of storage. This loss revealed earlier at low germination temperatures. However, the beneficial effect of presoaking was maintained during storage for at least 2 months.

4. Discussion and conclusion

The optimal temperature for germination of Valerianella olitoria seeds is c. 15°-20°C. This finding is valid for all commercial seed batches tested. At lower temperatures seeds are able to germinate perfectly, however the germination rate is reduced and the lag time to onset of germination is increased. Seed sensitivity to high temperatures varies between different cultivars and batches of each cultivar. The high temperature sensitivity can be compared to thermodormancy as described for various other species (Bewley and Black, 1982). Germination tests in the laboratory performed at non-optimal temperatures, can reveal better the seed heterogeneity and differences between various batches of seeds, differences which cannot be observed in tests at the optimal temperature. A better evaluation of seed quality and possible field emergence can be achieved when tests are carried out at non-optimal temperatures which are more similar to actual soil temperature at sowing time, either too low or too high.

Valerianella olitoria seeds are also very sensitive to oxygen deprivation. This phenomenon may explain the poor germination in soils with excess of water, or at too high temperature since oxygenation of the embryo decreases with increasing temperature (Côme, 1982).

Similar to other vegetable species, e.g. pepper (O'Sullivan and Bouw, 1984), celery (Heydecker and Gibbins, 1978), onion (Heydecker and Gibbins, 1978) and carrot (Heydecker and Coolbear, 1977; Corbineau and Côme, 1989), presoaking in water at suitable temperature markedly facilitates subsequent germination also of Valerianella olitoria seeds.

Such a treatment allows germination in a large range of temperatures and oxygen concentrations, and therefore might be very useful for improving and homogenizing field emergence under suboptimal conditions. However, although the beneficial effect of presoaking is maintained after redrying seeds, sowing must be done relatively rapidly after the treatment, since vigour and viability of presoaked seeds decrease progressively during storage.

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Table 1 - Germination percentage of seed batches of 3 cultivars at different temperatures. For each temperature germination was counted after 7 days. Mean of 4 measurements ± SD.

Cultivars	Batches (N°)	Germination (%) at 5°C	10°C	15°C	20°C	25°C
Jade	1	0	74 ± 8	90 ± 9	94 ± 3	90 ± 4
	2	0	50 ± 7	91 ± 4	92 ± 4	85 ± 7
Vit	1	0	30 ± 7	86 ± 6	87 ± 5	76 ± 9
	2	0	5 ± 4	72 ± 6	90 ± 3	84 ± 7
Verte de Cambrai	1	0	17 ± 4	93 ± 2	91 ± 4	82 ± 8
	2	0	5 ± 3	75 ± 4	68 ± 8	45 ± 9
	3	0	10 ± 8	92 ± 2	88 ± 6	48 ± 10

Table 2

Cultivar

Jade

Verte de Cambrai

Table 3

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Table 2 – Effect of different oxygen concentrations on germination at 20°C of one batch of the cultivar Jade and the cultivar Verte de Cambrai. Germinated seeds were counted after 5, 10 and 15 days in each oxygen concentration. Mean of 4 measurements ± SD.

Cultivars	Time of germination counts	Germination (%) in oxygen concentration of				
		3%	5%	10%	15%	21%
Jade	5 days	0	7 ± 2	31 ± 8	48 ± 6	61 ± 7
	10 days	3 ± 2	12 ± 6	59 ± 10	84 ± 3	86 ± 9
	15 days	9 ± 4	28 ± 7	70 ± 10	94 ± 2	95 ± 4
Verte de Cambrai	5 days	0	0	6 ± 4	53 ± 9	92 ± 3
	10 days	0	16 ± 5	85 ± 6	93 ± 4	99 ± 1
	15 days	2 ± 2	27 ± 7	86 ± 3	95 ± 3	99 ± 1

Table 3 – Effect of presoaking and redrying on germination of one seed batch of the cultivar Verte de Cambrai. Germination tests were carried out at 3 temperatures, and germinated seeds were counted after 5, 10 and 15 days. Presoaking was performed for 40 hours at 20°C, and germinability was tested without redrying or after redrying of seeds. Mean of 4 measurements ± SD.

Treatments	Time of germination counts	Germination (%) at		
		5°C	10°C	20°C
No treatment (control)	5 days	0	0	73 ± 10
	10 days	2 ± 2	89 ± 10	87 ± 11
	15 days	80 ± 6	93 ± 7	88 ± 9
40 hour-presoaking	5 days	30 ± 7	92 ± 5	90 ± 5
	10 days	93 ± 6	95 ± 4	91 ± 2
	15 days	97 ± 3	96 ± 2	92 ± 3
40 hour-presoaking + redrying	5 days	32 ± 8	90 ± 8	89 ± 7
	10 days	94 ± 4	96 ± 3	92 ± 5
	15 days	98 ± 2	97 ± 3	92 ± 3

Table 4 – Effect of presoaking followed by drying on the storage properties of one seed batch of the cultivar Verte de Cambrai. Germination was tested at 3 temperatures after different periods of storage at 20°C. Presoaking was performed for 40 hours at 20°C, and seeds were immediately redried. Germinated seeds were counted after 10 days at each temperature.

Treatment	Temperature of germination (°C)	Germination (%) after storage for (weeks)					
		0	3	6	8	15	26
No treatment (control)	5	0	0	0	0	0	0
	10	80	83	84	85	85	90
	20	98	97	98	97	98	97
40 hour-presoaking + redrying	5	97	91	87	85	60	10
	10	98	98	96	95	80	32
	20	98	98	96	95	90	38

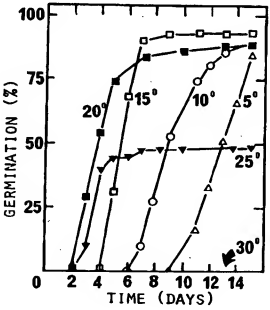

Figure 1 – Germination of one batch of seeds (Verte de Cambrai cultivar) at different temperatures, as a function of time.

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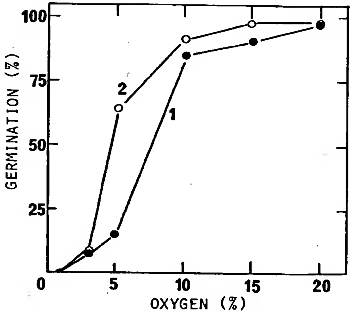

Figure 2 - Effect of oxygen concentration on the germination at 20°C of non-presoaked seeds (1) and of seeds presoaked for 40 hours at 20°C then redried (2). The seeds were from the same batch of Verte de Cambrai cultivar. Germinated seeds for each oxygen concentration were counted after 10 days.